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The physiological influence of 1-aminocyclopropane -1-carboxylic acid and Ethrel on phalaenopsis plantlets in vitro[1]

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ABSTRACT : Phalaenopsis plantlets in 50.0ml flask received 0, 2, 5, 10, 20 ppm Ethrel and 0.01, 0.1, 1 mM 1-aminocyclopropane-1-carboxylic acid (ACC) to study the physiological influence of Ethrel and ACC through culturese in vitro. Ethylene was produced by either the addition of Ethrel or ACC in flask. The treatment of 5. 0, 10.0 ppm Ethrel shortened and swelled the roots and produced vigorous plantlets which took the advantage of survival and less mechanical injury during transplant from flasks. However, 20ppm Ethrel induced overdosage effect. The treatment of 0. 1 mM ACC produced good quality plantlets as well as 5.0, 10.0 ppm Ethrel. The addition of 1 mM ACC caused small, curl leaf and shorter roots which presented the deteriorated effect on damage of roots and stunted growth of plantlets. The ACC content in different parts of plantlet was determined. The higher ACC residue was existed in root than in central young leaf and older leaves.

Key Words : Phalaenopsis, ethylene, 1-aminocyclopropane-1-carboxylic acid, Ethrel.

Introduction

In recent years, there has been increasing report that growth and differentiation of plant cell and tissues in vitro can be affected apparently by ethylene. This gaseous plant hormone was known to promote callus formation from culture citrus bud (Goren et al., 1979), to enhance plant regeneration from callus of *Nicotiana plumbaginifolia* and *Triticun aestivun* (Purnhauser et al., 1987), *Zea mays* (Songstad et al., 1988), promoted embryo production in anther cultures of *Brassica oleracea* (Biddington et al., 1988), and somatic embryogenesis of *Daucus carota* (Roustan et al., 1989). Babbar et al. reported that the ethylene precursor methionine (MET) and the ethylene releasing compound, Ethrel, stimulated embryo formation and plantlet development in *Datura metel* anther culture (Babbar et al., 1986). Cho and Kasha reported that the addition of ethylene promotors, Ethrel or ACC, increased embryogenesis (Cho and Kasha, 1989). Rio and Yang found that ethylene was inhibitory to

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root emergence and elongation of the cuttings (Rio and Yang, 1989). Lin found that the good quality plantlets which had more adventitious roots, no overgrown stem, no etiolated plantlets by the addition of 5, 10 ppm Ethrel (Lin, 1991). Since Adams and Yang demonstrated that the pathway of ethylene biosynthesis in apple fruit was as follow : MET → S-adenosylmethionine (SAM) → ethylene, (Adams and Yang, 1979), ethylene contributes to the microclimate which develops in the culture container during a culture cycle of plants (De Proft et al., 1985). This plant growth regulator is well known as an inducer of senescence and lignification (De Proft et al., 1987), and a regulator of a wide variety of phenomena in plant growth and development (Abeles, 1973, 1985., Yang, 1984).

In this study, phalaenopsis plantlets were cultured on agar medium with Ethrel and ACC content to investigate the ethylene production in vitro. These ethylene determinations were compared with some physiological responses for which ethylene is thought to be responsible. The primary purpose was to investigate the possible role of ethylene during plantlet growth before their transplant from flask. The conversion from ACC to ethylene will be examined to understand the ethylene biosynthesis through exogenous ACC in phalaenopsis plantlets in vitro.

Materials and Methods

Plant materials

Phalaenopsis (*Phalaenopsis* hybrid) plantlets with leaf of 1~1.5 cm in length were obtained from the node micropropagation in vitro.

The culture method

The plantlets were cultured in medium containing Murashige and Skoog salts (Murashige and Skoog, 1962) and the following constituents in mg/l : kinetin 1; NAA 2; thiamine · HCl 0.4; myo-inositol 100; sucrose 30,000; and Difco Bacto agar 7,000. Ethrel 2.5, 5, 10, 20 ppm or ACC 0.01, 0.1 or 1 mM was sterilized by millipore filter and added to media which had been sterilized by autoclaving 15 min, at 121°C. The pH in all media was adjusted at 5.7 before autoclaves. The media were dispensed into 50 ml flask at a rate of 20 ml. The flasks were sealed with autoclavable rubber septum. The plantlets wers maintained at constant 25 °C under 16 hr daily exposure at 5000 lux light of cool white fluorescent lamp F20T12CW General Electric Co. U.S.A., After 3 months the growth of roots and leaves of plantlets were recorded.

Ethylene measurement

The ethyene production of each different treatments was determinated to compare with some physiological responses for which ethylene was thought to be responsible. A 2ml air sample in flask was analyzed by using gas chromatograph equipped with flame ionization detector (Shimadzu gas chromatograph GC 8A) after the plantlets were placed on the medium in flask.

ACC assay

The portions of central young leaf, 2nd outward leaf, or roots weighing 1 g were extracted twice with 5 ml of boiling 88% ethanol respectively. Then, the ethanol with the extracted residue was evaporated under vacuum at 55°C. The residues were diluted in 2 ml of water. The ACC content in 0.3 ml aliquots of the aqueous solution was estimated according to the method of Lizada and and Yang (Lizada and Yang, 1979). The sample to be assayed was placed in a 10 ml test tube in an ice bath, 1 μmol HgClO was added by 100 μl, and the volume was brough to 0.4 ml. The tube was stoppered with a rubber serum cap. A cold mixture of 5% NaOCl and saturated NaOH (2 : 1, v/v) was injected into the tube with 100 μl volume mixture. The reaction mixture was reserved in ice for 5 to 10 min, the tubes were agitated vigorously, and the ethylene liberated was assayed by gas chromatograph.

Ethylene forming enzyme activity determination

Ethylene forming enzyme activity was assayed in vivo by measuring the conversion of administrated ACC to ethylene (Wang and Adams, 1982). Plant tissues weighing 2 g were immersed for 2 hr in a solution containing 3 ml of 1 mM ACC and 2 ml distilled water. Subsequently, the incubation solution was removed the plantlet tissues were separated by a high-frequency shaker, and after 4 hr in aerobic conditions the tissue was assayed for EFE activity by the production of ethylene. A 2 ml gas sample was assayed for ethylene by a gas chromatograph and the activity of EFE was determined by ethylene production.

Results and Discussion

The contribution of ethylene to the microclimate in the culture container is important. In culture containers the plantlets reacted differently in terms of ethylene and ACC after different treatments. When phalaenopsis plantlets were cultured in a sealed container with media containing Ethrel or ACC, their leaf blades hardly expanded slowly (Table 1). The influence was proportional to Ethrel concentration or ACC concentration. The overgrown size plantlets were cultured in the medium without Ethrel and ACC (Fig. 1). However, the leaf blades of plantlets which cultured in 1 mM ACC curled up under unexpectedly (Fig. 2). This phenomenon was found in lily, tulip (Abeles, 1973), gerbera (Lin, 1991). The leaf number was less affected by Ethrel, but slightly affected by ACC treatment. Both Ethrel and ACC treatments showed less change in root number (Table 2). Results from enriching Ethrel and ACC to culture system have been contradictory with stimulation (Robbins et al., 1983), suppression (Geneve and Heuser, 1983), or no effect (Batten and Mullins, 1978) on root number being reported by different laboratories. The uncertainty concerning ethylene involvement is compounded by results with species and crops. Nevertheless, Abeles reported that exogenous ethylene suppresses root elongation and increase radical espansion (Abeles, 1973). The results were same as a number of investigators have observed on suppression of root elongation and root radical expansion (Smith and Robertson, 1971; Fujino et al. 1988). The plantlets which cultured in Ethrel 5 ppm (Fig. 3) and ACC 0.01 mM medium did not show curled leaf and deteriorated effects on roots by ethylene (Fig. 4).

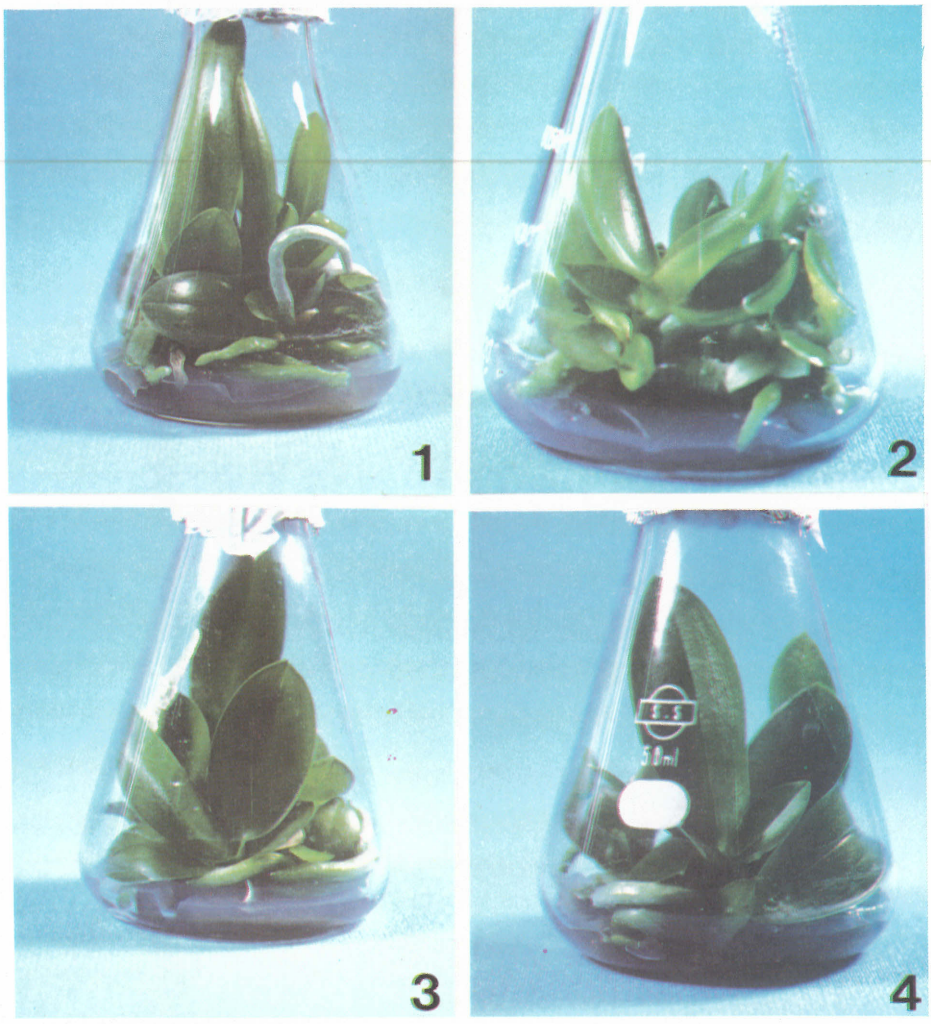

Fig. 1. The phalaenopsis plantlets cultured in vitro without Ethrel and ACC grew long root and overgrown leaves.
Fig. 2. ACC 1mM caused the curled up leaves of phalaenopsis plantlets in vitro culture.
Fig. 3. The well grown plantlets grew well in the medium with Ethrel 5 ppm after 3 months cultured in vitro.
Fig. 4. The phalaenopsis plantlets grew well in the medium with ACC 0.01 mM after 3 months cultured in vitro.

Table 1. The effect of Ethrel and ACC on leaf growth of phalaenopsis plantlets after 3 months subcultured from young mericlone plantlets.

Treatment		leaf number	leaf length (cm)	leaf width (cm)
Control		4.4±0.7*	4.2±0.6	1.3±0.4
Ethrel	2.5ppm	4.3±0.6	3.9±0.2	1.2±0.3
	5.0	4.2±0.5	3.8±0.7	1.3±0.4
	10.0	4.1±0.3	3.7±0.4	1.3±0.3
	20.0	4.0±0.4	3.3±0.6	1.2±0.2
ACC	0.01mM	3.8±0.6	3.8±0.7	1.1±0.1
	0.1	3.6±0.4	3.6±0.8	1.1±0.4
	1	3.4±0.7	2.4±0.4	0.9±0.2

* Values represent the mean ± SE of 5 replcates.

Table 2. The effect of Ethrel and ACC on root growth of phalaenopsis plantlets after 3 months subcultured from young mericlone plantlets.

Treatment		root number	root length (cm)	root width (cm)
Control		4.6±0.4*	2.6±0.3	2.5±0.4
Ethrel	2.5ppm	4.5±0.8	1.8±0.4	2.6±0.3
	5.0	4.4±0.6	1.7±0.3	3.3±0.6
	10.0	4.2±0.8	1.5±0.5	3.0±0.4
	20.0	3.6±0.4	1.4±0.4	2.8±0.5
ACC	0.01mM	4.2±0.7	1.8±0.7	2.6±0.7
	0.1	4.1±0.5	1.7±0.3	3.2±0.6
	1	4.0±0.4	1.6±0.4	3.4±0.3

* Values represent the mean ± SE of 5 replicates.

Ethylene production and EFE activity from th addition of Ethrel

Ethylene is a gas with limited solubility under physiological conditions. Many studies on ethylene production and action have been carried out in vitro using tissue slices, organ segments, or whole plantlets placed in vessels that are sealed to contain ethylene. (Tisserat and Murashige, 1977). The cumulative ethylene released from plantlets was proportional to Ethrel concentration in the nutrient medium (Table 3). The accumulated for 4 weeks ethylene was 42 nl/l for Ethrel 12.5 ppm; 86.4 nl/l for 5 ppm; 92.3 nl/l for 10 ppm; 130.2 nl /l for 20 ppm and 7.2 nl/l for control. Ethrel could be attributed to a volatile component, ethylene production. The EFE activity which contributed to ethylene production showed the correspondence of Ethrel proportion concentration (Table 3). The EFE activity was assayed in vivo, and was determined by the amount of ethylene production from the plant leaf discs including ACC as substrate. In terms of EFE activity of plantlets which cultured in Ethrel medium increased depending on the increase of Ethrel concentration. Yang et al. demonstrated that enzyme catalyzing conversion of ACC to ethylene is constitutive in plant tissue (Yang and Hoffman, 1984). Consequently, the autocatalytic rise in ethylene production and constitutive characteristics of EFE were also found in this studies.

Table 3. Ethyene production and EFE activity in phalaenopsis plantlets treated with Ethrel in vitro after subcultured 4 weeks.

Treatment		Ethylene concentration (nl/l)	EFE activity (nmol C_2H_4/g F.W.H)
Ethrel	0 ppm	7.2±0.7*	0.7±0.1
	2.5ppm	42.3±0.4	3.6±0.4
	5.0	86.4±0.6	7.7±0.2
	10.0	92.3±0.4	8.8±0.3
	20.0	130.2±0.7	11.2±0.2

* Values represent the mean ± SE of 5 replicates.

Ethylene production and EFE activity from the addition of ACC

Yang and Hoffman have elucidated that ACC is an intermediate in the pathway of ethylene biosynthesis from methionine is well established (Yang and Hoffman, 1984). Bradford and Yang discovered that ACC accumulated in the anaerobic root and then was transported via the xylem to the shoot where ACC was converted to ethylene under aerobic condition, caused petiole epinasty and other morphological responses (Bradford and Yang, 1980). In these studies, a progressive increase of ethylene was accompanying with increasing concentration of ACC (Table 4). The increasing of ethylene also associated with increasing EFE activity (Table 4). In plant tissue, the ACC uptake through roots repespondence with ethylene production due to EFE constitutive enzyme property. The formation of ACC from S-adenosylmethionine (SAM) by the enzyme ACC synthase and the conversion of ACC to ethylene by the ethylene forming enzyme (EFE) system have been shown to be important steps in the control of ethylene production (Yang, 1980). Chalutz et al. discovered that the increased conversion of ACC to ethylene may be mediated through a change in the membrance milieu of EFE (Chalutz at el., 1984). Manning found that EFE activity was stimulated 27 fold, approaching the rate for control petals given ACC (Manning, 1986). In these studies, the progressive increase of EFE activity and ethylene production were also proportion to ACC concentration due to the addition of ACC and EFE property.

Table 4. Ethyene production and EFE activity in phalaenopsis plantlets treated with ACC in vitro after subcultured 4 weeks.

Treatment	Ethylene concentration (nl/l)	EFE activity (nmol C_2H_4/g F.W.H)
ACC 0 mM	7.2±0.7*	0.7±0.1
0.01	12.4±0.4	1.0±0.4
0.1	14.3±0.6	1.5±0.3
1.00	36.5±0.8	2.7±0.2

Values represent the mean ± SE of 5 replicates.

The EFE system is labile, membrane-associated and regulated by carbon dioxide, ethylene, and also other hormones (Manning, 1986). The ethylene production, regulation and physiological influence on plantlets through the application of ACC, Ethrel in vitro still need to do further work. Perhaps future studies of EFE function and regulation will give unambiguous identification of the protein associated with ethyelne forming activity, making it possible to develop tools for the study of these very important enzyme system at the molecular level.

The content of ACC in different plant parts cultured in vitro

The different content of ACC was found in different plantlet parts (Table 5). The roots accumulated the highest concentration, then, central young leaf and outer old leaf for each treatment. Moreover, the residues of ACC content in different plantlet parts were proportional to the application of ACC concentration. Bradford and Yang pointed out that ACC can be synthesized in one organ and transported to another site where it express activity

through conversion to ethylene (Bradford and Yang, 1980; De Proft et al., 1987). In this study, the application of ACC 1 mM showed deteriorated effect (Fig. 2) but addition of ACC 0.01 mM represented the favorable good quality plantlets (Fig. 4) were same as the addition of Ethrel 5 ppm worked (Fig. 3). In conclusion, this studies demonstrated that the responses of Ethrel and ACC shortened, swelled the roots and leaves were in the involvement of ethylene. The application of Ethrel and ACC should be considered by using the optimum dosage to prevent plantlets from deteriorated effect of ethylene.

Table 5. ACC content in different plant parts of phalaenopsis plantlets with the addition of ACC in vitro after subcultured 2 weeks.

plant part / treatment		ACC (nmol/g F.W.) central young leaf	2nd outer leaf	root
ACC	0 mM	0.8±0.1*	0.2±0.0	1.5±0.2
	0.01mM	5.4±0.4	1.1±0.3	11.2±0.6
	0.1	6.4±0.6	1.3±0.4	12.3±0.4
	1.00	9.8±0.4	2.1±0.6	16.5±0.7

* Values represent the mean ± SE of 5 replicates.

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環氨基丙烷羧酸及益收素對試管內蝴蝶蘭苗之生理影響[1]

林 瑞 松[2]

蝴蝶蘭幼苗於50毫升三角瓶內以0，2.5，5，10，20 ppm 的益收素 (Ethrel) 及0.01，0.1，1 mM 的環丙烷氨基羧酸 (1-aminocyclopropane-1-carboxylic acid, ACC) 以研究二者對蝴蝶蘭幼苗之生理影響。二者均可產生乙烯。試驗結果顯示以益收素 5.0 及 10.0 ppm 可以使根部縮短肥大，植株健旺，有利於瓶苗移植減少操作時機械傷害。但是益收素濃度在 20 ppm 時則有藥劑過量現象。以 0.1 mM 的環丙烷氨基羧酸亦得到與益收素 5.0 及 10.0 ppm 處理效果一樣優良瓶苗，處理濃度在 1 mM 時造成葉片變小、捲葉、根短、顯示了有害於根部及阻礙生長的現象。環丙烷氨基羧酸之含量分別於不同部位檢測，根部留存量較高於葉片部分。

關鍵詞：蝴蝶蘭，乙烯，環氨基丙烷羧酸，益收素。

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